Journal of Food Protection, Vol. 73, No. 2, 2010, Pages 366–371

Research Note

Evaluation of a Direct-Fed Microbial Product Effect on the Prevalence and Load of *Escherichia coli* O157:H7 in Feedlot Cattle†

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MS 09-356: Received 24 August 2009/Accepted 14 October 2009

ABSTRACT

Direct-fed microbials (DFM) have been identified as potential preharvest interventions for the reduction of foodborne bacterial pathogens such as *Escherichia coli* O157:H7. This study evaluated the efficacy of a DFM consisting of *Bacillus subtilis* strain 166 as an antimicrobial intervention strategy for the reduction of prevalence and load of *E. coli* O157:H7 in feces and on hides of feedlot cattle. Cattle ($n = 526$) were divided among 16 feedlot pens. Half of the pens received the DFM, and the other half did not. Hide and fecal samples were collected from each animal on days 28, 63, and 84 of the feeding trial. Over the course of the 84-day feeding period, there were no significant differences observed between treatments for either hide or fecal prevalence of *E. coli* O157:H7, or for the percentage of animals that were shedding *E. coli* O157:H7 at high levels (≥200 CFU/g) in their feces or harboring *E. coli* O157:H7 at high levels (≥40 CFU/cm^2) on their hides. In addition, there was no significant difference between the average daily gains for the treated and control groups, with both groups averaging 1.3 kg/day. We concluded that the DFM tested would not be an effective preharvest intervention against *E. coli* O157:H7.

Much of the research to date on reducing *Escherichia coli* O157:H7 from the food supply has been focused on the postharvest side of the production chain. Several postharvest antimicrobial interventions (i.e., cattle or carcass rinse with various antimicrobials, steam vacuuming, steam pasteurization, etc.) have been shown to be efficacious in reducing levels of foodborne pathogens on beef carcasses and in the subsequent ground product *(2, 4, 7, 11, 12, 14, 19)*. Preharvest interventions do not share the same wealth of techniques that have been approved for use against foodborne bacterial pathogens.

A great deal of research effort is currently focused on development and validation of effective preharvest interventions *(10, 22, 31)*. One area of research on preharvest interventions pertains to direct-fed microbials (DFM). DFM (also referred to as *probiotics*) have been identified as potential preharvest interventions *(8, 10, 27, 34, 36)*. DFM are hypothesized to function by a variety of mechanisms (for example, competitive exclusion, immune modulation, or bactericidal activity via production and secretion of bacteriocins) to remove the target organism from the intestinal tract of the animal *(20)*. For a DFM to be a useful antimicrobial intervention in the beef industry, it must act against *E. coli* O157:H7, a foodborne pathogen found to colonize the intestinal tracts of cattle. By reducing the *E. coli* O157:H7 population in the intestinal tract of feedlot cattle, the amount of *E. coli* O157:H7 shed in feces would be reduced, thereby potentially reducing the risk of carcass contamination by reduction of fecal-to-hide contamination at the feedlot and/or reduction of the contamination of the lairage environment at processing plants, which has been linked to carcass contamination at processing *(1)*. This study evaluated the efficacy of a *Bacillus subtilis*–based DFM as an antimicrobial intervention strategy for the reduction of prevalence and load of *E. coli* O157:H7 in feedlot cattle.

MATERIALS AND METHODS

Strain. The DFM consisted of *B. subtilis* strain 166 made into a premix by Ivy Animal Health (Overland Park, KS). The particular strain had been isolated from corn silage and initially characterized as exhibiting broad-spectrum inhibition against gram-negative bacteria. This strain was further analyzed via in vitro experiments and found to possess small, molecule-mediated bactericidal activity against *E. coli* O157:H7. The premix contained *B. subtilis* strain 166 at 6.4×10^8 CFU/lb ($\sim 2.9 \times 10^8$/kg) with inactive carrier ingredients, 70% ground limestone (wt/wt) and 29.5% rice hulls (wt/wt).

Feed. The DFM premix was added to the normal feed ration (50 lb [~22.7 kg] of premix to 7,500 lb [~3,402 kg] of feed). All animals were fed twice a day. The feeding procedure consisted of the animals in the control pens being fed the normal ration. The

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† Mention of trade names or commercial products in this article is solely for the purpose of providing specific information and does not imply recommendation or endorsement by the U.S. Department of Agriculture.

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TABLE 1. *Ration ingredients*[a]

Ration	Corn	Corn silage	Protein supplement[b]	MGA premix[c]
Growing	29.5	66	4.5	0
Intermediate 1	51.39	39.83	4.5	5.61
Intermediate 2	65.22	26.38	4.5	4.78
Intermediate 3	72.1	17.5	4.5	4.16
Finishing	78.95	12.75	4.5	3.8

[a] Cattle were stepped through three intermediate rations over a 2-week period, and then fed the finishing ration for the duration of the test. Values are expressed as percentage of dry matter.
[b] Protein supplement contained Rumensin at 408 g/T of feed.
[c] MGA, melengestrol acetate.

treated pens were then fed the ration plus DFM. The treatment was added at a level of 1.25×10^8 CFU of *B. subtilis* strain 166 per animal per feeding, so that each animal received on average 2.5×10^8 CFU/day. A dedicated feed truck was used for the DFM-containing feed mix to prevent cross-contamination of feed. The cattle were stepped through three intermediate rations over a 2-week period (Table 1), and then fed the finishing ration for the duration of the test. The final ration feed ingredients on a percentage of as-fed basis were 62% cracked corn, 30% corn silage, 5% protein supplement, and 3% melengestrol acetate premix. The protein supplement contained Rumensin at 408 g/T. Melengestrol acetate was added to a level of 0.04% as fed.

Animals and pens. Sixteen pens (50 by 250 ft [~15.2 by 76.2 m]) were utilized for this study. Prior to entry of the cattle, feed bunks and water troughs were cleaned and sanitized. Each pen housed approximately 32 animals. Half of the pens ($n = 8$) received the DFM in the ration, while the other pens received the normal ration. The DFM ration was fed over a period of 84 days, from May to August of 2007. Animals were screened for *E. coli* O157:H7 on hides and in feces the week before initiating the experiment. Animals were assigned to pens based on pathogen status (prevalence and level of *E. coli* O157:H7). Control and treated pens were interspersed in pairs, with pens sharing water troughs in the same group, either control or treated. Control and treated pens were adjacent to each other, and animal contact may have occurred.

Sample collection. Cattle were weighed on days 0, 28, 63, and 84 of the experiment to determine average daily weight gain. On these same days, fecal and hide samples were collected as follows: rectal feces grab samples were collected from each animal by rectal palpation, and hide samples (1,000 cm^2) were collected from each animal behind the left shoulder by using sterile, moistened Speci-Sponges (Nasco, Fort Atkinson, WI).

***E. coli* O157:H7 enumeration.** *E. coli* O157:H7 was enumerated from hide and fecal samples by using a Spiral Plater (Spiral Biotech, Norwood, MA), following the protocol developed by Brichta-Harhay et al. *(9)*. Limits of detection for the enumeration assay were 200 CFU/g and 40 CFU/100 cm^2 for the fecal and hide material samples, respectively.

***E. coli* O157:H7 prevalence.** Samples were processed according to methods previously described, with slight modifications *(5, 6)*. PCR was used to confirm that each isolate harbored genes for the O157 antigen, H7 flagella, and at least one of the Shiga toxins *(18)*.

***Salmonella* analyses.** The presence of *Salmonella* was determined with enrichment and isolation procedures developed previously *(6, 9, 26, 35)*.

Statistical analysis. For each trait, pen means and prevalences were calculated, and ''pen'' served as the experimental unit. For each trait and each sampling time, one-way analysis of variance was conducted with PROC GLM (SAS 9.1, SAS Institute, Inc., Chicago, IL).

RESULTS AND DISCUSSION

Probiotics containing *B. subtilis* have been used in human health applications as well as in the production of agricultural animals such as poultry, sheep, and swine *(13, 17, 21, 27, 30)*. Benefits to human health from such probiotics have been reported for gastrointestinal disorders including diarrhea, inflammatory bowel disease, lactose intolerance, and *Helicobacter, Salmonella,* or *Shigella* infections *(24, 29)*. Swine fed a *Bacillus*-based DFM had a higher rate of average daily gain than did control animals *(13)*. Similarly, poultry fed *B. subtilis* as a growth promoter were reported to have feed conversion rates comparable to those fed antibiotic growth promoters *(27)*. Fritts et al. *(16)* reported both improved performance and decreased pathogen prevalence on carcasses for broiler chickens treated with Calsporin, a commercially marketed *B. subtilis* DFM. The levels of aerobic plate counts, coliforms, and *Campylobacter* and the prevalence of *Salmonella* were reduced significantly on broiler carcasses of the treated birds *(16)*. There are little data regarding the anti–*E. coli* O157:H7 or growth promoting properties of DFM containing *B. subtilis* when used in cattle, and even less of DFM composed solely of *B. subtilis*.

Hide and fecal samples were obtained from 512 heifers 1 week prior to pen assignment and the start of the DFM feeding period. The fecal and hide prevalences for *E. coli* O157:H7 on day −7 were 24.3 and 88.1%, respectively (Table 2). At this point, there were 23 animals shedding *E. coli* O157:H7 at high levels (≥200 CFU/g of feces), and 26 animals that harbored high concentrations of *E. coli* O157:H7 (≥40 CFU/100 cm^2) on their hides. Shedding status was blocked across treatments to evenly distribute among treatments and pens the animals shedding *E. coli* O157:H7.

TABLE 2. *Effect of DFM on* Escherichia coli *O157:H7 in feces and on hides of feedlot cattle*[a]

Day	Feces enumeration[b]				Feces prevalence				Hide enumeration[c]				Hide prevalence			
	Control	DFM	SEM	$P > F$	Control	DFM	SEM	$P > F$	Control	DFM	SEM	$P > F$	Control	DFM	SEM	$P > F$
−7	4.7	4.3	0.6	0.64	24.6	23.9	0.5	0.31	4.3	5.9	1.3	0.40	85.9	90.2	2.3	0.22
0	4.3	2.0	1.0	0.12	16.8	10.2	1.9	0.03	1.6	1.2	0.6	0.64	71.1	31.6	2.9	<0.0001
28	3.9	0.8	1.3	0.11	12.1	12.1	4.7	1.00	1.2	3.5	2.2	0.47	34.4	32.0	9.5	0.86
63	5.1	5.5	2.0	0.89	14.5	12.9	5.4	0.84	19.9	5.1	7.4	0.18	45.7	29.3	11.2	0.32
84	13.7	10.6	3.5	0.54	28.9	22.7	6.5	0.51	21.1	17.2	8.7	0.76	83.2	78.1	5.8	0.55

[a] Control, DFM, and SEM values are percent positive. DFM, direct-fed microbial (*B. subtilis* strain 166).
[b] Feces enumeration (percent positive) = the number of animals shedding *E. coli* O157:H7 at levels of ≥200 CFU/g of feces for a 10-g sample divided by the total number of animals in the treatment.
[c] Hide enumeration (percent positive) = the number of animals harboring *E. coli* O157:H7 at levels of ≥40 CFU/100 cm^2 divided by the total number of animals in the treatment.

In spite of efforts to minimize initial variation in *E. coli* O157:H7 prevalence and levels across treatments, the day 0 sampling period was the only sampling period in which significant differences were detected. Both hide and fecal prevalences for the control animals were significantly higher than were the hide and fecal prevalences for the treated animals at day 0. DFM feeding had not occurred at this time and was not the cause of the differences in *E. coli* O157:H7 prevalence. For days 28, 63, and 84, there were no significant differences detected for either hide or fecal prevalence of *E. coli* O157:H7 (Table 2). It has been shown that antimicrobial interventions may be effective by reducing pathogen levels rather than pathogen prevalence *(2)*. An intervention can reduce the bacterial load by several log cycles, but not eliminate the pathogen entirely. This would lead to a significantly reduced foodborne illness risk, without a reduction in prevalence of the organism. Reducing the fecal, and subsequently hide load, is the main objective of preharvest intervention *(23)*.

Preharvest interventions are not expected to eliminate foodborne disease–causing microorganisms from the cattle population, but they do need to reduce the hide levels to below the threshold capacity of the antimicrobial interventions in a beef processing plant. Currently, U.S. beef processing plants typically employ multiple-hurdle intervention schemes to minimize the risk of carcass contamination *(3)*. Each combination of antimicrobial interventions has some upper limit of bacterial load that can be removed from the beef carcass as it is processed. If this threshold limit is exceeded, then the finished product may be contaminated. The role of preharvest intervention is to reduce the pathogen load coming into the plant to below this threshold. However, the DFM evaluated in this study did not reduce the bacterial load, as the percentages of control or treated animals that were shedding *E. coli* O157:H7 at high levels in their feces or harboring *E. coli* O157:H7 at high levels on their hides were not different. At the individual animal level, the range of levels of *E. coli* O157:H7 being shed was similar, with animals from both treated and control groups exceeding 10^5 CFU/g (Table 3). As would be expected, based on these data, the range of levels of *E. coli* O157:H7 found on the hides of animals also were similar (Table 4). The data from this experiment lead to the conclusion that the *Bacillus*-based DFM tested would not be an effective preharvest intervention against *E. coli* O157:H7. Concerning *Salmonella* prevalence and levels, there were not sufficient observations of *Salmonella* to enable substantive conclusions to be made.

Although the DFM premix was tested for potency both prior to the start of the trial and at the completion of the trial, the DFM premix was mixed thoroughly with the feed ration in the feed truck before dispensing in the feed trough, and little to no feed was left in the trough unconsumed, it should be noted that fecal samples were not analyzed for *B. subtilis* at any time during the study. Therefore, the results must be interpreted cautiously with regard to the possibility that the animals, either collectively or individually, did not receive the DFM. The authors believe this to be an unlikely

TABLE 3. *Enumeration of* Escherichia coli *O157:H7 from fecal samples*[a]

	Day 0		Day 28		Day 63		Day 84	
CFU/g	Control	Treated	Control	Treated	Control	Treated	Control	Treated
200–999	10	3	5	1	5	5	11	9
1,000–9,999	1	1	2	1	6	5	10	6
10,000–99,999	0	1	3	0	0	1	5	7
100,000–999,999	0	0	0	0	2	1	5	4
1,000,000–9,999,999	0	0	0	0	0	0	3	1

[a] Number of animals shedding *E. coli* O157:H7 at the indicated concentration.

TABLE 4. *Enumeration of* Escherichia coli *O157:H7 from hide samples*[a]

	Day 0		Day 28		Day 63		Day 84	
CFU/100 cm^2	Control	Treated	Control	Treated	Control	Treated	Control	Treated
40–99	3	2	2	7	36	12	36	27
100–999	1	1	1	2	12	1	14	14
1,000–9,999	0	0	0	0	3	0	4	2
10,000–99,999	0	0	0	0	0	0	0	0
100,000–999,999	0	0	0	0	0	0	0	1

[a] Number of animals harboring *E. coli* O157:H7 on hides at indicated concentration.

scenario, but encourage the reader to be mindful of this point in their consideration of the results.

Studies using *Lactobacillus*-based DFM have reported reductions in *E. coli* O157:H7 prevalence in cattle feces. Tabe et al. *(34)* reported a 32% reduction in *E. coli* O157:H7 detection when steers were fed a combination of *Lactobacillus acidophilus* (LA 51) and *Propionibacterium freudenreichii* (PF 24). While the DFM tested was effective in preventing colonization, it did not improve the probability that an animal would stop shedding once colonized with *E. coli* O157:H7 *(34)*. Other groups using *L. acidophilus* (NP 51) have reported reductions in the *E. coli* O157:H7 fecal prevalence of cattle ranging from 35 to 49% *(8, 27)*. Stephens et al. *(32)* observed reductions in the *E. coli* O157:H7 prevalence on cattle hides when the animals were fed a DFM combining *L. acidophilus* (NP 51) and *P. freudenreichii* (PF 24).

It is not known at this time why the DFM tested herein did not exhibit the same anti–*E. coli* O157:H7 activity observed in vitro when fed to cattle. Two possible explanations focus on the idea that the majority of *E. coli* O57:H7 was not exposed to the small-molecule inhibitor produced by the DFM strain. In the first scenario, as the DFM was fed in spore form, there may not have been sufficient time for germination of the cell and expression of the inhibitor prior to the DFM strain being excreted from the animal. In the second scenario, *E. coli* O157:H7 would not have been in contact with the inhibitor because *E. coli* O157:H7 was concentrated at the recto-anal junction colonization site *(25),* while the *B. subtilis* strain was either concentrated at another site in the bovine gastrointestinal tract or remaining passively diffuse in the contents that pass through the gastrointestinal tract. In either situation, the inhibitor would not reach a significant concentration in close proximity to the *E. coli* O157:H7 population colonizing the animal. Other possible explanations require further study to elucidate the mechanistic details that led to these results.

Aside from pathogen mitigation, DFM have been shown to provide performance advantages in agricultural animals. In feedlot cattle, supplementation with lactic acid bacteria have produced mixed results regarding performance enhancement. Several studies *(8, 15, 28)* have reported no significant effect on animal performance, while others *(20, 33)* have observed increases in daily gain and feed efficiency. In this study, there was no significant difference between the average daily gains for the treated and control groups, with both groups averaging a 1.3-kg increase per day (Table 5). Based on these results, the *B. subtilis* strain 166–based DFM did not affect animal performance.

TABLE 5. *Effect of DFM on animal performance, i.e., daily gain*

Day	Control (kg)	Treated (kg)
0	421	425
28	462	462
63	498	496
84	527	531
Avg daily gain	1.26 A[a]	1.25 A

[a] Average daily gain values in the same row that share a common letter are not different ($P > 0.05$).

ACKNOWLEDGMENTS

Partial funding for this project provided by Ivy Animal Health, Inc. We thank Julie Dyer, Emily Griess, Bruce Jasch, Dee Kucera, Frank Reno, Sarah Schumacher, and Greg Smith for technical support, and Marilyn Bierman for secretarial support.

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